Effects of Therapeutic Ultrasound and Aussie current associated with High-Intensity Interval Training on abdominal adiposity in overweight and obese young adults: Protocol for a Randomized Control Trial

Ana Carolina Aparecida Marcondes Scalli, Patricia Rehder-Santos, Étore De Favari Signini, Alex Castro, Carla Cristina Dato, Leonardo Furlan, Richard Eloin Liebano, Aparecida Maria Catai

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Abstract

Background: More than half of the world's population will be overweight or obese by 2035, and it is known that physical exercise, such as high-intensity interval training (HIIT), is a tool for controlling obesity by improving body composition and metabolic profile. Non-invasive techniques, such as therapeutic ultrasound (TUS) and the Aussie current, have shown potential in controlling adipose tissue, but their effects combined with HIIT remain unknown.

Objective: This study aims to evaluate the effects of TUS+Aussie combined with HIIT on body composition, serum metabolic profile and cardiovascular autonomic modulation (CAM) in obese individuals.

Methods: This is a randomized, double-blind (researcher and outcomes assessor) clinical study. The study was approved by the Human Research Ethics Committee of the Universidade Federal de São Carlos (number: 7.389.480 and CAAE: 64624522.8.0000.5504). The participants will be randomized into three groups: active TUS+Aussie group associated with HIIT (AG), placebo group for TUS+Aussie associated with HIIT (PG) and TUS+Aussie only group (HG). Primary outcomes include changes in subcutaneous adipose tissue thickness, body composition, and serum metabolic profile. Secondary outcomes assess perceived stress, body image, blood biochemistry, sleep quality, and cardiovascular autonomic modulation (CAM). Data analysis involves linear mixed models estimated using the maximum likelihood method with an appropriate covariance matrix structure.

Results: A total of 60 participants will be recruited and randomized between February 2024 and June 2025. The baseline assessments and intervention are scheduled to be completed in August 2025, and data collection will be completed by the end of September 2025. Data management is still ongoing, therefore data analysis has not yet been carried out.

Conclusions: This is the first study to combine TUS+Aussie with HIIT, potentially integrating the effects of lipolysis and fat oxidation and possible changes in the serum metabolic profile and CAM. The results could optimize treatment duration, promote changes in lipid profile and maintain cardiovascular health in people with obesity. Clinical Trial: EnsaiosClinicos.gov.br RBR-4xh6232 (UTN: U1111-1287-2345).

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ABSTRACT

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Trial Registration: EnsaiosClinicos.gov.br RBR-4xh6232 (UTN: U1111-1287-2345).

KEYWORDS
combined therapy; abdominal subcutaneous fat; obesity; lifestyle; physiotherapy.

BACKGROUND

According to a report by the World Obesity Federation, more than half of the world's population will be overweight or obese by 2035[1]. Excess abdominal fat is a primary determinant for developing insulin resistance and other metabolic disorders [2]. Lifestyle modification is the most recommended approach for treating and preventing obesity at any stage of life [2]. In addition to exercise and a controlled diet, non-invasive techniques have emerged as safe and effective tools for managing adipose tissue [3; 4]. These techniques include different electrophysical agents, such as low-intensity laser [5], cryotherapy [6], radiofrequency [7], infrared radiation [8] and therapeutic ultrasound (TUS) [4; 9].

TUS is a therapeutic modality widely used in physiotherapy clinical practice [10; 11]. The non-linear propagation of TUS waves induces mechanical forces on particles at the applied site, resulting in biological effects [4], including pyroptosis [12]. This term refers to a form of programmed cell death mediated by caspase-1 or caspase-11, characterized by the secretion of pro-inflammatory cytokines [12; 13]. Cells undergoing pyroptosis exhibit mitochondrial membrane potential loss, deoxyribonucleic acid fragmentation, nuclear condensation, and the release of cytoplasmic contents into the bloodstream, including fatty acids [13; 14].

TUS can be combined with Aussie electrical current to stimulate the lymphatic system, producing a strong but comfortable paresthesia [11; 15]. The Aussie current is characterized by a medium-frequency alternating wave modulated at a low frequency and is indicated for various applications, including muscle strengthening, pain control, lymphatic drainage and edema control [11]. The combined effects of these electrophysical resources – TUS and Aussie current – alongside aerobic exercise post-therapy, may enhance fat β-oxidation. However, despite promising results for cellulite reduction with the combination of TUS and Aussie current [16; 17; 18], prior studies were limited by small sample sizes, lack of a placebo group, and insufficient focus on post-therapy clinical outcomes, such as serum metabolic changes [18]. In addition, other forms of intervention have been widely suggested for reducing body composition and promoting significant metabolic changes in obese individuals, such as high-intensity interval training (HIIT). HIIT alternates between periods of high intensity and active recovery and has become a popular training method due to its time efficiency.

Although previous studies[19; 20] have shown better effects of HIIT on the aerobic capacity of patients with cardiovascular disease when compared to continuous moderate-intensity training, the effects of HIIT on cardiovascular autonomic modulation (CAM) in people with obesity are poorly understood. A systematic review evaluated the effects of HIIT on CAM, based on six studies that used heart rate variability as a measure[21]. The results showed that HIIT can improve autonomic modulation by

increasing parasympathetic and reducing sympathetic cardiac modulation in healthy individuals and patients with metabolic syndrome, making it a promising strategy for cardiovascular rehabilitation[21].

As for the effects of HIIT on body composition, another review analyzed body fat percentage, fat mass and fat-free mass in cycling, running on the ground and running on a treadmill[22]. The findings showed that all forms of HIIT significantly reduced fat mass (-1.86 kg) and body fat percentage (-1.53%) compared to the control group [22].

Although previous studies have reported reductions in abdominal circumference and body composition following TUS and Aussie application [3; 4; 10; 11], none have evaluated this technique in combination with HIIT in overweight or obese participants. In addition to optimizing time, the combination of both interventions also aims to optimize the breakdown, drainage and oxidation of fat, promoting weight loss. There is a need for clinical trials to investigate the potential clinical effects of this therapeutic modality in humans, as suggested by animal models [17; 18; 23].

The results of this study will determine whether TUS and Aussie therapy induce changes in body composition, manage serum metabolic profile and improve CAM indices.

OBJECTIVES

This pragmatic study aims to evaluate the effects of ten sessions of combined therapy (TUS+Aussie) alone and combined with HIIT on body composition, serum metabolic profile and cardiac autonomic modulation in people with obesity. The study also investigates the impact of these interventions on perceived stress, body image and sleep quality in people with obesity.

METHODS

Trial design

This is a randomized, pragmatic, placebo-controlled, double-blinded, three-arm (1:1:1) clinical trial. The protocol was developed in accordance with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines [24].

Study setting

This research will be conducted at the Universidade Federal de São Carlos (UFSCar), in the Cardiovascular Physiotherapy Laboratory/Physical Exercise Research Nucleus (LFCV/NUPEF) within the Department of Physiotherapy.

Participants will be recruited through social media advertisements. An electronic form hosted on Google Forms® will be shared via Instagram®. Interested participants who meet the basic inclusion criteria will be contacted via WhatsApp® to provide additional information about the study and assess their eligibility. Participants who do not respond to the messages will receive up to three phone call attempts at different times. If these contact attempts are unsuccessful, the individual will be considered unreachable and ineligible for the study.

Eligibility Criteria

Table 1 - Inclusion and exclusion criteria

Inclusion criteria	**Exclusion criteria**
Men and women	Current smokers, alcoholics, illicit substance users, or ex-smokers within the past 12 months
Ages 18–40 years at enrolment	History of body mass reduction treatments using drugs or electrophysical agents in the last 12 months
Classified as overweight or having grade 1/2 obesity according to the Fat mass index (FMI), as measured by Dual Energy X-ray Absorptiometry (DXA)	Electrocardiogram abnormalities, including ischemia, overloads, conduction disorders, serious arrhythmias such as ventricular tachycardia.
Healthy adults, without any chronic or acute disease. Starting any physical activity in the last three months.	Presence of any of these conditions or diagnoses of diseases: neurological, renal, respiratory, cardiac, musculoskeletal, venous thrombosis, anticoagulant therapy and/or bleeding disorders, active cancer or cancer treatment in the last five years, conditions or lesions in the abdominal region, diabetes mellitus type I or II, hypothyroidism or hyperthyroidism and current pregnancy in the last 12 months

Informed consent

This study adheres to the ethical principles outlined in the Declaration of Helsinki. The research protocol was approved by the Research Ethics Committee of the UFSCar (approval number [CAAE no. 64624522.8.0000.5504]) and was registered in the Brazilian Registry of Clinical Trials on February 8, 2023, and updated on October 2, 2024 (RBR-4xh6232 - UTN: U1111-1287-2345). Participants will be informed of their right to withdraw from the study at any time without penalty or loss of benefits. Additionally, all collected data will be treated with strict confidentiality and used solely for research purposes.

Before any data collection, the outcome assessor obtains written informed consent from participants. Only those who provide consent proceed to the baseline evaluation and randomization.

Randomization and blinding

In this study, the researchers responsible for the evaluations and those analyzing the data will be blinded. To ensure the feasibility of blinding, the blinded research teams will not participate in the interventions, and all information will be coded to prevent the identification of participants and intervention groups. The effectiveness of blinding will be assessed using a questionnaire, completed by the evaluators at the end of the final assessment for each data collection block.

After the initial assessments, participants will be stratified (age range, sex, and fat mass index (FMI)) and randomly assigned to one of three groups. Participants in Group 1 (AG) will undergo combined therapy (TUS + Aussie) along with the HIIT protocol. Participants in Group 2 (PG) will undergo placebo combined therapy (TUS + Aussie) with devices turned off, along with the HIIT protocol. Participants in Group 3 (HG) will receive only the combined therapy (TUS + Aussie) without the HIIT protocol.

For stratified randomization, participants will be initially grouped into blocks based on similar characteristics, including age range (20–29 years or 30–40 years), sex (male or female), and FMI (overweight, grade I obesity, or grade II obesity). These characteristics will be chosen to control potential confounding factors. Within each block, participants will be then randomized into the three study groups using Microsoft Excel®, ensuring an unbiased allocation process. Block sizes were not

fixed, further reducing the risk of allocation predictability.

The randomization process will be conducted by a researcher designated exclusively for this task, who will have no contact with the study participants, or the researchers involved in data collection. This researcher will also be responsible for generating random alphanumeric codes to ensure the anonymity of each participant's information.

Evaluations

Baseline evaluation

Subjects who meet the eligibility criteria will be considered eligible for randomization and will have a baseline evaluation scheduled.

To characterize the sample, the initial assessment records information, such as sex, age, anthropometric data, family history, medication use, education, occupation and comorbidities.

Study assessments and timeline

All study participants will have to attend two face-to-face visits: one at baseline (week - 1) and one at the final assessment (week 6). In addition, participants will be assessed by a nutritionist and receive nutritional guidance and diet plans (week 0). The treatment will last 5 weeks. A detailed schedule is available in Table 2, respectively.

Table 2 – Study period

	STUDY PERIOD							
	Enrolment	**Allocation**	**Post-allocation**					**Close-out**
TIMEPOINT**	***- 4 w***	**0 w**	***1 w***	***2 w***	***3 w***	***4 w***	***5 w***	***6 w***
ENROLMENT:								
Eligibility screen	X							

Informed consent	X							
Nutritional Evaluation	X							
Allocation		X						
INTERVENTIONS:								
AG								
PG								
HG								
ASSESSMENTS:								
SAT	X	X						X
Body Composition	X	X						X
Metabolic profile			X				X	
Perimeter	X	X						X
Blood Test	X	X						X
Questionnaires	X	X						X
Exercise test		X						
24-hour food recall			X	X	X	X	X	
CAM	X	X	X				X	X

Schedule of enrolment, interventions, and assessments subcutaneous adipose tissue (SAT), CAM = cardiovascular autonomic modulation, AG = Active TUS+Aussie group associated with HIIT, PG = placebo group for TUS+Aussie associated with HIIT, HG = TUS+Aussie only group.

Initial Evaluation

Initial information (lifestyle and inclusion criteria) will be collected online using a screening form link for men and women.

Body mass and height measurements will be taken with a calibrated scale. Participants will be advised to be barefoot and wear light clothing. Height is measured with the participant standing and using a stadiometer.

A member of the research team (ACAMS) is responsible for carrying out all study assessments, as well as screening and recruiting participants, minimizing bias.

Abdominal subcutaneous adipose tissue

Ultrasound is used as the main endpoint to assess abdominal SAT [4]. Previously, intra-rater reliability was assessed [25]. A GE Healthcare Venue 40® Ultrasound (model NZCART, GE Medical Systems, Ltd, city Wuxi, Changjiang Road, China) is used. Subcutaneous fat, defined as the distance in centimeters between the dermis and the external surface of the fascia of the abdominal muscles, is measured using a linear transducer with a frequency of 12 MHz placed transversely one cm above and one cm below the umbilical scar [26; 27]. Participants will be examined in the supine position, without any artifact compressing the abdominal region, with their knees bent and instructed to breathe in and out. Three measurements will be taken, each at the end of an exhalation, with minimal pressure on the abdominal cavity for greater measurement precision [28; 29].

Body Composition

To determine total body mass, fat mass percentage per region, and visceral fat, we use dual-energy X-ray absorptiometry (DXA, Hologic Discovery A, Bendford, MA). This assessment is considered the gold standard for evaluating body composition in overweight and obese individuals [30]. In addition, DXA is used to classify participants as overweight or obese (grade I and II) [31].

Participants must fast for four hours, refrain from vigorous exercise for at least 12 hours, avoid caffeine and alcohol for the previous 24 hours and consume a normal meal the night before the test. They should not wear any metal objects or accessories during the test to prevent interference with the total body composition results. The participant is positioned in a supine position, with internal rotation of the thighs, legs, and feet, and instructed not to speak or move during the examination. The results obtained will be transmitted to a computer connected to the equipment, where the report shows the data in grams and percentages and which will be used to analyze total lean mass (kg), total bone mass (%), total fat mass (g), mass/stature² (kg/m²), fat mass in the android and gynoid regions (g), visceral fat (g) and, through calculations, to deduce subcutaneous fat [32; 33].

Serum metabolic profile

Venous blood samples will be collected in the cubital fossa by a specialized professional, after fasting for three hours, following a standardized meal. Blood samples will be taken three times: before and immediately after the TUS+Aussie session and immediately after HIIT. Each tube of blood collected will be left to stand at room temperature for 30 minutes. They will then be centrifuged at 1450×g for 10 minutes (Sorvall ST 8 Benchtop Centrifuge, Thermo Scientific, Massachusetts, USA). The serum is divided into three aliquots in 1.5 ml microtubes using 5 ml disposable pipettes and stored immediately at -80 °C until further analysis. The serum samples will then be thawed at the UFSCar Chemistry Department and sent for analysis using the hydrogen nuclear magnetic resonance technique. Serum glycerol, total cholesterol (TC), very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides will be assessed. These will be measured using wet chemistry (except for LDL, which will be calculated using the Friedwald equation) (Advia 1800, Siemens, Germany). The experimental procedures for this analysis will follow those described in Signini et al. [34] and Castro et al. [35].

Cardiovascular Autonomic Modulation

To ensure the quality of the electrocardiogram (ECG) signal collection, the recommendations suggested by Catai et al. will be followed. [36]. All participants will be instructed to have a regular night's sleep the night before, avoid alcoholic and caffeinated drinks, refrain from strenuous physical exercise at least 24 hours before and on the day of the assessment and avoid heavy meals up to 2 hours before the assessment. All procedures will be explained beforehand to familiarize participants with the equipment and the assessor. The experiment will be carried out in the afternoon, in a quiet room, with the minimum number of people in the LFCV, with controlled temperature and relative humidity (22-23ºC, 40-60%, respectively) [36]. Before starting data collection, the participants will rest for 10 to 15 minutes to stabilize the signals [36]. After this, they will remain in the supine position for 15 minutes and then perform active postural change, remaining in the orthostatic position for 15 minutes. Participants will be instructed to breathe spontaneously and not to move or speak during the experiment.

RR intervals (RRi) will be captured by ECG (BioAmp FE132, ADInstruments, Australia) from the

MC5 lead. In addition, respiratory movements will be captured using a respiratory belt positioned around the participants' chest (Marazza, Monza, Italy) to obtain the respiratory rate during the test. Arterial pressure (AP) will be measured continuously, beat-to-beat, by a photoplethysmograph (Finometer PRO; Finapress Medical System, The Netherlands) on the middle finger of the right hand. RRi signals, AP and respiratory movement will be sampled at 1kHz using a commercial acquisition device (PowerLab 8/35; ADInstruments).

Classical linear indices of heart rate variability will be calculated in the time and frequency domains. The indices calculated in the time domain will be the standard deviation of the NN interval (SDNN), which represents sympathetic and parasympathetic modulation together, and the square root of the mean of successive NN intervals (RMSSD), which indicates parasympathetic modulation [37; 38]. After detecting the QRS complex on the ECG, the apex of the R wave will be identified using parabolic interpolation. The heart period (HP) will be calculated as the temporal distance between two consecutive parabolic apexes. A stable sequence of 256 beats (for systolic AP [SAP] and HP) will be chosen in the supine and orthostatic positions, and if isolated ectopic beats are present, they will be linearly interpolated [39]. From these 256 points, the mean and variance of HP (μHP and σ 2 HP), the mean and variance of SAP (μSAP and σ^2 SAP) will be calculated in the time domain.

The variability parameters of HP and SAP will be evaluated according to the autoregressive model [39]. The spectral components will be broken down into low frequency (LF from 0.04 to 0.15 Hz) and high frequency (HF >0.15 to 0.4 Hz) reported in absolute units. The power of the spectral components will be expressed in absolute units (HFabs) or normalized units (LFnu) [38].

Symbolic analysis will be used to evaluate the HP and SAP series as defined in Porta et al.[40]. This technique is based on the 6-level uniform quantization procedure applied to HP and SAP series, which transforms series into sequences of symbols (from 0 to 5) from which patterns of 3 consecutive symbols will be constructed[41]. All possible patterns will be grouped, without loss of information, into families according to the number and type of variations between subsequent symbols: i) 0V pattern: all symbols are the same; ii) 1V pattern: two underlying symbols are the same and the rest are different; iii) 2LV pattern: the two variations between adjacent symbols have the same sign; iv) 2UV pattern: the two variations between adjacent symbols have opposite signs. Percentages of patterns within each experimental session were evaluated and denoted with 0V%, 1V %, 2LV% and 2UV%, respectively. The 0V% index will be understood as a marker of sympathetic modulation when calculated in the HP and SAP series and the 2UV% index will be understood as an index of vagal modulation when calculated in the HP series [40; 42; 43].

The baroreflex system will be evaluated by cross-spectral analysis using a bivariate autoregressive

parametric approach [44]. The time series relationships between AP and HP will be represented as coherence, phase and transfer function gain. The coherence function shows the degree of association between the HP and SAP variabilities ($K^2_{HP\text{-}SAP}$) [44; 45]. Its values range from 0 to 1, where values closer to 1 represent better coupling between the signals. The phase represents the temporal relationship between the series, i.e. the delay between the change in the SAP signal resulting from the heartbeat ($Ph_{HP\text{-}SAP}$) and will be evaluated in the HF ($Ph_{HP\text{-}SAP}$ HF) and LF ($Ph_{HP\text{-}SAP}$ LF)[44].

Sleep quality

Sleep quality is assessed using the Pittsburgh Sleep Quality Index (PSQI) for sleep quality, adapted for Brazilians by Bertolazi et al. [46]. It is a reliable instrument (ICC = 0.65) [47] that assesses seven components of sleep: subjective quality, sleep latency, sleep duration, sleep efficiency, sleep disturbances, medication use, and daily dysfunction. For each component, the score ranges from 0 to 3, and the sum gives a maximum score of 21. Scores above five indicate poor sleep quality.

Anxiety, depression, and stress

Anxiety and depression will be assessed using the Hospital Anxiety and Depression Scale (HADS), validated for Brazilians (32). It consists of 14 items divided into two domains (depression and anxiety) with seven items each. The items have a 4-point Likert scale (from 0 to 3), resulting in total scores ranging from 0 to 21 for anxiety and depression. The cut-off points indicating moderate to severe symptoms are anxiety domain ≥ 8 and depression domain ≥ 9.

Stress is assessed using the Depression, Anxiety and Stress Scale (DASS-21), which measures the levels of these disorders based on behaviors experienced in the last seven days. The items use a 21-question Likert scale, which is translated into Brazilian Portuguese, to rate the frequency or severity of the participants' experiences on a four-point scale [48].

Body Image

The Body Image Questionnaire (BSQ), validated for the Brazilian population (30), is a self-administered questionnaire consisting of 34 items designed to measure satisfaction and concerns about body shape. It is organized on a six-point scale: 1 never, 2 rarely, 3 sometimes, 4 frequently, 5 very frequently, and 6 always. The score is the sum of the answers, where ≤ 110 points indicates no concern, ≥ 111 or ≤ 138 points indicates mild concern, ≥ 139 or ≤ 167 points indicates moderate concern, and if the sum of the answers is greater than 168 points, it indicates serious concern [49]. This questionnaire helps assess potential changes in satisfaction or concern about the body after the proposed treatment.

Biochemical blood tests

Participants will be referred to a specialized clinic for blood collection. They will be instructed to fast for 10 hours before the test. The following will be collected complete blood count; glycated hemoglobin (by high-performance liquid chromatography); fasting glycemia; insulin resistance (by enzymatic and chemiluminescent immunoassay); evaluation of the homeostatic model (HOMA-R); C-reactive protein (by immunoturbidimetry); homocysteine (by chemiluminescence); total cholesterol; high-density lipoproteins (HDL); low-density lipoproteins (LDL) and triglycerides (by enzymatic method). The tests will be clinically relevant in the context of obesity and abdominal adiposity and may contribute to future studies, to determine whether any changes occur in these variables after the intervention. The results will be reported by the Unit's doctor and sent to the researcher.

Perimetry

The waist-to-hip ratio is a calculation based on waist and hip circumference measurements [50]. It is often used to assess the risk of developing diseases such as high cholesterol, diabetes, and high blood pressure [50]. The presence of these cardiovascular risk factors, associated with increased abdominal fat, leads to a high risk of diseases such as myocardial infarction, stroke, hepatic steatosis, and mortality. The values considered normal for males and females are < 0.95 cm and < 0.80 cm, respectively [50]. The waist-to-height ratio, on the other hand, is an index that assesses health risks by indicating the proportionality between waist circumference (a possible indicator of central fat

accumulation) and height, suggesting a risk of cardiovascular or metabolic diseases [51]. Values below 0.5 cm indicate a lower risk of diseases, while values above 0.5 cm indicate a higher risk [51]. The measurements will be taken with the participant standing upright, wearing light clothing. The waist circumference is measured as the average distance between the last floating rib and the anterior superior iliac crest [52]. The hip circumference is measured around the widest part of the buttocks, at the level of the greater trochanter of the femur [52]. All measurements will be taken twice using an inextensible tape measure, and if there is a difference of three cm, a third measurement is taken for both waist and hips [52]. The ratio between the measurements is calculated as the waist circumference divided by the hip circumference.

Neck circumference is measured using an inextensible tape measure, perpendicular to the longitudinal axis of the neck, over the thyroid cartilage [53]. Participants will be in an anatomical position, either standing or sitting, with their head in the Frankfurt plane, shoulders relaxed, and in inspiratory apnea [53]. The cut-off points for overweight and obesity will be 37 cm for men and 34 cm for women [54]. The values above these indicate an increased risk of cardiovascular risk factors [54].

Physical Activity

All participants will be characterized by their level of physical activity at a single point in time (prior to the intervention). The *International Physical Activity Questionnaire* (IPAQ) long version is used. The IPAQ is a validated instrument for the Brazilian population that assesses the level of physical activity. It has 27 questions related to physical activities performed in a week at different intensities: light, moderate, and vigorous, lasting at least 10 minutes continuously. The questions are covered in four dimensions: work, transportation, domestic activities, and leisure, as well as the time spent seated [55]. The score is calculated by adding the workload for each sub-item separately [55]. This questionnaire helps classify the level of physical activity before the proposed interventions.

Before the intervention, *a treadmill ergometric test* is conducted using the Ellestad protocol (48), for clinical cardiological assessment and to assist in prescribing HIIT. The test is interrupted according to the criteria recommended by the III SBC Ergometry Guidelines [56]. The original Borg scale is used to assess the participant's perception of dyspnea, lower limb fatigue, and angina during exercise. This scale ranges from 0 to 10, where 0 is no dyspnea and 10 is the most intense dyspnea, fatigue and angina [57]. The following determines the presence of maximum or peak effort: peak heart rate (HR_{PEAK}) ≥ 85% of that predicted for age (220 - age) and Borg's perceived exertion of 18 on the 6-20

scale, patient exhausted [58].

INTERVENTIONS

Nutritional

The nutritional intervention will be conducted by an experienced nutritionist. All participants receive nutritional guidance 30 days before the combined therapy and HIIT. This initial intervention aims to adjust macronutrient intake, optimize energy consumption, and improve diet quality by reducing the consumption of ultra-processed foods and increasing minimally processed foods. Macronutrient adjustments follow the recommendations of the Dietary Reference Intakes (DRIs), with carbohydrates comprising 45-65%, proteins 10-35%, and lipids 20-30% of the total caloric intake [59].
Following this phase, participants undergo a new series of assessments and then begin five weeks of combined therapy and HIIT. During these five weeks, dietary control is monitored by the nutritionist to ensure consistency in dietary patterns throughout the study. Daily energy intake is considered stable if variations remain below 10% during the protocol.
Food intake is assessed weekly using a 24-hour recall, a questionnaire that quantifies all food and drink consumed in the previous 24 hours, the link is sent to the participant and is self-applicable once a week during the 5 weeks of training. Total energy intake and macronutrient composition will be calculated using the Dietbox® program (https://dietbox.me/pt-BR). Energy requirements will be estimated based on the DRIs [60]. For qualitative analysis, foods will be categorized according to their processing level - natural, minimally processed, culinary ingredients, processed, and ultra-processed - as defined by the 2014 Food Guide for the Brazilian population [61].

Therapy Combined Application Time Calculation

Before the first session, the application time for the combined therapy will be individually determined. Measurements will be performed by the same blind assessor using an inelastic tape measure with the participant in a standing position. The distance between the last rib (floating rib) on both sides (x) and the distance between the anterior inferior iliac crests (y) will be measured. The area of the abdominal region, determined by the product

$x \times y$, is then divided by 18. This divisor corresponds to the area of the head used in the therapy, which is made up of three spheres, each with a diameter of 6 cm. Dividing this total area by the value 18 allows us to determine the time needed to apply the therapy to each participant, according to the formula:

$$\text{Application time} = \frac{x \times y}{18}$$

Pre-Training Instructions

Before each training session, participants will be instructed to i) wear comfortable clothing and exercise-appropriate footwear; ii) ensure adequate sleep the previous night; iii) hydrate with water two hours before the session and bring a personal water bottle; iv) avoid strenuous physical activity and alcoholic consumption within 24 hours prior to the session.
Food consumption will also be recommended 24 hours before the first and last sessions, as described in the *Nutritional* section.

Session Scheduling and Follow-Up

To minimize data loss, participants receive reminders about the date and time of each session. Physiotherapists responsible for interventions may also call participants, if necessary, to confirm appointments. For evaluations and re-evaluations, the blind evaluator contacts the participants by telephone. At the end of the 10 treatment sessions, the re-evaluation is scheduled between three and a maximum of 15 days after the last session.

Group 1: TUS and Aussie with HIIT (AG)

Participants in AG will be treated with TUS+Aussie (Sonofocus® Ibramed, Indústria Brasileira de Equipamentos Médicos EIRELI - Amparo, SP, Brazil). The protocol combines continuous ultrasound (3 MHz; 2 W/cm^2) and Aussie current (1 kHz; modulated at 10 Hz). The participant will be instructed that the sensation must be tingling and comfortable (sufficient intensity to produce strong

but comfortable paresthesia, without stimulation to painful paresthesia) [62]. In every session and every five minutes, the therapist will record the current intensity of the Aussie current and evaluate the levels of paresthesia on a scale of 0 to 10, with 0 (no tingling sensation) and 10 (maximum tolerable tingling). Given the possible habituation to the paresthesia caused by the Aussie current, the scale will be applied every five minutes during the session, and a score of five will be set as the threshold for adjusting the intensity of the current, i.e. tolerable/supportable tingling.

The treatment is applied to the abdominal region using ultrasound. The therapist ensures consistent pressure and maintains slow, slightly circular, horizontal movements without losing contact with the surface.

In the final session, participants complete a printed questionnaire that includes the following: i) reported intensity of the Aussie current; ii) "Would you recommend this therapy to someone else?"; iii) "Do you believe you received the active treatment, the placebo or are unsure? Why?". Also in the last session, the blinded therapist will answer a questionnaire with questions about which group they believe each participant was allocated to and the reason for this choice.

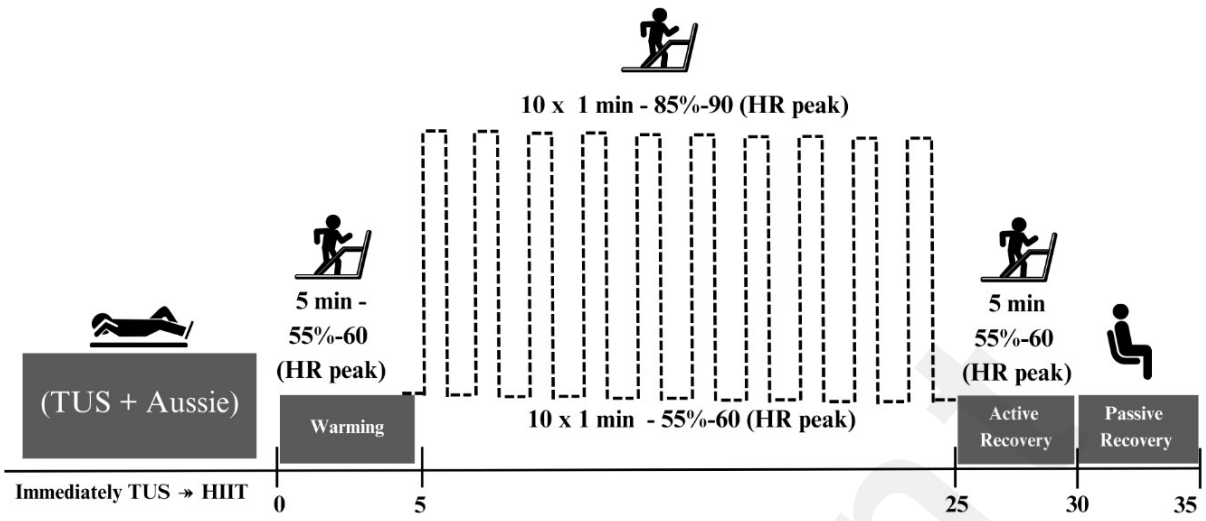
Immediately after the TUS+Aussie application, participants will take to the treadmill for the HIIT session. Participants will be instructed to maintain proper posture with relaxed shoulders, a forward-facing stance, and a breathing pattern of inhaling through the nose and exhaling through the mouth, avoiding apnea during the exercise. The Borg scale (6-20) [63] will be applied 10 seconds before each load change during the 20-minute interval protocol.

The heart rate is continuously monitored using Polar H10 attached to an elastic chest strap positioned at the xiphoid process. This Polar is paired via Bluetooth with a tablet.

The HIIT protocol lasts 30 minutes and includes:

- *Warm-up:* Five minutes, progressively reaching 55%-60% of the peak heart rate (HR_{PEAK}) determined in the ergometric test.
- *Interval phase:* 10 cycles of one-minute high-intensity interval (85-90% of HR_{PEAK}), interspersed with 10 cycles of one-minute light-to-moderate intensity (55%-60% of HR_{PEAK}). After five sessions, the load will be updated by 5-10% considering physiological adaptations (60-65% HR_{PEAK} and 90-95% HR_{PEAK}).
- *Cool-down and recovery:* Five minutes of active cool-down, followed by passive recovery with the participant seated (figure 1).
- *Guidelines and organization*: Controlled temperature and humidity (21-24ºC and 40-60%), prior hydration with water and afternoon.

Figure 1.



Summary of the high-intensity interval training protocol following the application of combined therapy, along with the progression of the intervention protocol. TUS = therapeutic ultrasound; HIIT = high-intensity interval training; HR_{PEAK} = peak heart rate during the ergometric test.

Group 2: TUS and Aussie placebo with HIIT (PG)

All the participants in this group will follow the same procedures and sequence as in Group 1. However, PG participants will receive placebo treated with TUS and Aussie (Sonofocus® Ibramed, Indústria Brasileira de Equipamentos Médicos EIRELI - Amparo, SP, Brazil). The protocol combines continuous ultrasound (0 MHz; 0 W/cm^2) and Aussie current (0 kHz; modulated at 0 Hz). Immediately after the TUS+Aussie treatment, all participants proceed to the treadmill to start the HIIT, following the same protocol as AG (figure 2).

Group 3: TUS and Aussie without HIIT (HG)

Participants in HG receive the same treatment as TUS+Aussie applied in AG. However, after the application of TUS+Aussie, it will not be suitable for HIIT training.

OUTCOMES

The primary outcomes involve a comparison (between groups) of the effect of 10 sessions of

combined therapy (TUS+Aussie) alone vs. in combination with HIIT, specifically on the impact of abdominal subcutaneous adipose tissue thickness, body composition, metabolic profile and serum glycerol release. Secondary outcomes assess the impact on cardiovascular autonomic modulation, sleep quality, anxiety, depression, body image concerns and blood biochemical variables (table 3).

Table 3 - Primary and secondary outcomes of the study

Variable	Description
Primary outcomes	
Abdominal subcutaneous adipose tissue (cm)	Ultrasound imaging [4; 25]
Body composition (Kg, % and g)	Dual-energy X-ray absorptiometry [31]
Metabolic profile	Venous blood collection Hydrogen nuclear magnetic resonance [34; 64]
Secondary outcomes	
Cardiovascular autonomic modulation	Heart Rate and Arterial Pressure Variability[36; 38; 40; 41]
Sleep quality (score)	Pittsburgh Sleep Quality Index [46; 47]
Anxiety, depression and stress (score)	Hospital Anxiety and Depression Scale [65] Depression, anxiety and stress scale [48]
Body image (score)	Body shape questionnaire [49]
Blood tests	Complete blood count, glycated hemoglobin, fasting glucose, ultra-sensitive C-reactive protein, $cholesterol_T$, high density lipoprotein, ow density lipoprotein and triglycerides
Perimeter measurements (cm)	Waist-to-hip ratio, Waist-to-height ratio, Neck circumference and Waist circumference

Monitoring adverse events

There is a possibility that participants may experience side effects in response to the study protocol. Regarding HIIT, intense shortness of breath, generalized tiredness and dizziness may occur, and this will be recorded in the study. In addition, as the study involves blood sampling, there is a possibility of damage not directly related to the intervention. The expected adverse effects, such as bruising and local pain, will be monitored. Any adverse effects other than these will be recorded and reported, although such occurrences are considered highly unlikely. During assessments and sessions, participants will also be asked about their well-being and whether they have experienced any changes or symptoms since the start of the study. This information will be documented to ensure ongoing safety and monitoring of participants, even if a specific adverse event classification is not applicable in this study.

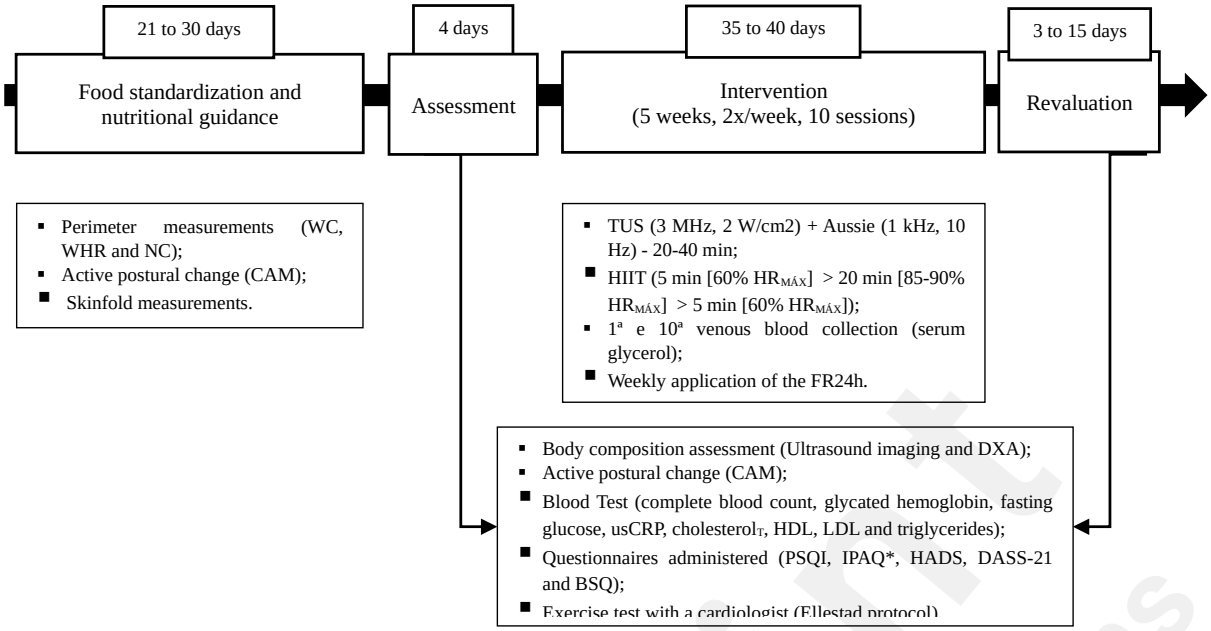


Figure 2 - Flowchart of assessments before, during and after 10 intervention sessions.
Legend: WC = waist circumference; WHR = waist-hip ratio; NC = neck circumference; CAM = cardiovascular autonomic modulation; TUS = therapeutic Ultrasound; HIIT = high-intensity interval training; $HR_{MÁX}$ = maximum heart rate; FR24h = 24-hour food recall; DXA = dual-energy x-ray absorptiometry; usCRP = ultrasensitive c-reactive protein; HDL = high density lipoprotein; LDL = low density lipoprotein; PSQI = Pittsburgh sleep quality; IPAQ = International physical activity questionnaire (*characterization); HADS = Anxiety and depression assessment scale; DASS-21 = Depression, anxiety and stress scale; BSQ = Body image questionnaire.

STATISTICAL ANALYSIS

Sample size

The primary outcome, “percentage of body fat”, was used to calculate the required sample size using the RStudio software 12.1, assuming a moderate expected effect size $f \geq 0.15$, a significance level of 5% and to promote a statistical power of 80%. Assuming an estimated mean of 33.5%, 35.5%, and 37% for AG, PG, and HG, respectively, at the end of the intervention period, a correlation of 0.4 between the pre- and post-intervention measures, and a standard deviation of 3.5% for all groups, a total of 18 participants per group is necessary to achieve 80% statistical power with the alpha level of 5%. To account for a 10% attrition rate, the target sample size was increased to 20 participants per group.

Descriptive statistics and baseline data

A Consolidated Standards of Reporting Trials (CONSORT) style flowchart [66] will present the number of patients selected and all reasons for exclusion prior to randomization. Demographic and socioeconomic information, including age, sex, race, socioeconomic status and education level, as well as baseline clinical characteristics such as body mass index, glycated hemoglobin, smoking status, alcohol consumption, and comorbidities, among other variables, will be described by study arm and overall. Categorical variables will be reported as absolute numbers and percentages. Continuous variables with a reasonably symmetric distribution will be summarized as means and standard deviations. For highly skewed continuous variables, median and interquartile ranges will be used.

Statistical methods for primary and secondary outcomes

Data analysis will be conducted by a researcher blinded to the participants' allocation to their respective groups. The data will be entered into an Excel spreadsheet for subsequent analysis.

For quantitative variables, the assumption of normality of data distribution and homogeneity of variances will be checked using the Shapiro–Wilk test and Levene test, respectively. To compare participant characteristics at baseline between groups, one-way analysis of variance (ANOVA) or Kruskal-Wallis test will be applied when appropriate. For comparisons between groups involving categorical variables and the Chi-square test or Fisher Exact test will be used. To compare primary and secondary outcomes between and within groups, linear mixed models (LMM) estimated using the maximum likelihood method with an appropriate covariance matrix structure will be used, assuming Group [TUS+Aussie group associated with HIIT (AG), placebo group for TUS+Aussie associated with HIIT (PG), and TUS+Aussie only group (HG)], Moment (pre and post intervention), and/or Time (baseline, post TUS+Aussie, and post HIIT), and interactions (Group–Moment, Group-Time, Moment-Time, Group-Moment-Time) as fixed factors and participants as a random factor [64]. In addition, Moment and Time will be assumed as a repeated-measure effect. The model that fits the data will be the one with the minimum Akaike information criterion. When appropriate, the data may be subjected to a Box-Cox transformation to better adhere to a normal distribution [67; 68]. Whenever a significant F value is obtained in the ANOVA or LMM, a Sidak post hoc adjustment for the purpose of pairwise multiple comparisons will be applied. The significance level will be set at 5% ($p < 0.05$). All analysis will be conducted using PASW statistics software version 25.0 (SPSS, Chicago, IL,

USA),

RESULTS

The recruitment and testing phase began in February 2024. The study is scheduled to be completed by the end of August 2025. Data analysis will begin in September 2025, and the results are expected to be published by 2026.

DISCUSSION

Implications

The scientific literature lacks studies that evaluate the effects of therapeutic ultrasound combined with Aussie current, associated or not with HIIT. A controlled, randomized experimental design will be used that follows the SPIRIT guidelines (23), and which describes the parameters used to achieve the best results for the population studied.

To the best of our knowledge [4; 9; 11; 15; 18], this study will be the first clinical trial to compare the effects of therapeutic ultrasound combined with Aussie current and HIIT in overweight, grade I or II obesity participants. The findings will provide evidence regarding the effects or lack thereof of this therapeutic approach. Furthermore, as a randomized clinical trial, it will contribute to systematic reviews, fostering new evidence syntheses and addressing existing knowledge gaps.

Contributions to the area

This study aims to guide health professionals to design more effective interventions, whether through combined therapy alone or associated with HIIT, for overweight, grade I or II obesity participants. By determining whether the combination of two therapies (TUS+Aussie and HIIT) is more effective than just one of them in treating or preventing the disease. In addition, the results will also contribute

to patients' knowledge of non-pharmacological and non-invasive treatment for obesity and whether the proposed guidelines and interventions will be significantly different in the primary outcomes (reduction in abdominal SAT, change in body composition and blood biochemical variables). In fact, if the results show significant differences between the proposed interventions, obese patients will benefit from the combination of two non-invasive and fast-acting therapies. In addition, studies have been cited with durations ranging from three to fifteen weeks, thus this study will be able to verify the results of five weeks of intervention[22]

Prospects for future research

While this study will focus on the impact of combining two interventions on overweight, grade I or II obesity participants, future research could explore comparisons with other exercise protocols, such as high-intensity resistance exercise. This is particularly relevant given that different exercise modalities elicit distinct musculoskeletal adaptations, which can lead to varied changes in body composition.

Strengths and weaknesses of the study

This is a randomized, double-blind, placebo-controlled study designed in accordance with the most rigorous standards for conducting and processing data in clinical trials, ensuring the reliability of the results. It is important to highlight that the intervention period was defined to assess whether the intervention protocol, considering the usual recommendations for exercise and diet, as well as the number of TUS+Aussie application sessions commonly offered by aesthetic clinics, results in fat reduction for patients with obesity. Thus, this study will provide healthcare and aesthetic professionals with scientific evidence to understand the mechanisms involved in applying the studied therapies, expected results, and potential systemic impacts. To achieve these objectives, the study includes several outcome measures, including subcutaneous adipose-tissue thickness, body composition, profile metabolic and serum glycerol release, allowing for a comprehensive evaluation

of the combined effects of TUS+Aussie and HIIT. Additionally, by incorporating secondary outcomes such as cardiovascular autonomic modulation, sleep quality, and perceived stress, the study offers a broader understanding of the physiological and psychological effects of the intervention.

Despite these strengths, the study presents some limitations. The sample size, although adequate for preliminary findings, may not be large enough to detect differences in cardiovascular autonomic modulation results. In addition, individual variations in adherence to the HIIT protocol and lifestyle factors, such as diet and physical activity outside the study, can influence the results. However, it is important to note that strict guidelines are provided to minimize these influences, ensuring greater control over external variables and the reliability of the findings. Another potential limitation is the study duration; while the intervention period is sufficient to observe short-term changes, the long-term effects on adiposity and metabolic health remain unknown. Furthermore, the study population consists of overweight and obese individuals, which may limit the generalizability of the findings to other populations, such as those with severe obesity or metabolic disorders.

In summary, this study will provide a robust framework for evaluating the combined effects of TUS+Aussie and HIIT on body composition, cardiovascular autonomic modulation, and cardiorespiratory and metabolic health. Despite its limitations, the findings will contribute valuable insights into non-invasive strategies for obesity management and may support future research and clinical applications.

TRIAL STATUS

This protocol (December 2024 version) corresponds to an ongoing study that began recruiting in February 2024 and is scheduled for completion in August 2025.

AVAILABILITY OF DATA AND MATERIALS

Additional information, including the dataset, statistical code, written consent form, and detailed protocol information, will be made available upon request to the corresponding author following the study`s completion. The data will be made available after the study has been published.

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DECLARATIONS

Ethical approval and consent to participate

This study was approved by the Research Ethics Committee of the Federal University of São Carlos (opinion number: 7.389.480), whose guidelines are in accordance with the Declarations of Helsinki. All participants will take part in this study after signing the Free and Informed Consent Form.

Consent for publication

Not applicable.

Conflicts of Interest

The authors declare that they have no conflicting interests.

ABBREVIATIONS

AG = Ative TUS+Aussie group associated with HIIT

AP = Arterial Pressure

AUSSIE = Aussie current

BSQ = Body image questionnaire

CAM = cardiovascular autonomic modulation

CAPES = Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

CNPq = Conselho Nacional de Desenvolvimento Científico e Tecnológico

CONSORT = Consolidated Standards of Reporting Trials

DASS-21 = Depression, anxiety and stress scale

DRIs = Dietary Reference Intakes

DXA = dual-energy x-ray absorptiometry

ECG = Electrocardiogram

FAPESP = Fundação de Amparo à Pesquisa do Estado de São Paulo

FMI = Fat Mass Index

FR24h = 24-hour food recall

HADS = Anxiety and depression assessment scale

HDL = high density lipoprotein

HG = TUS+Aussie only group

HIIT = high-intensity interval training

HOMA-R= Homeostatic model assessment

HP = Heart Period

HR_{PEAK} = peak heart rate

IPAQ = International physical activity questionnaire

LDL = low density lipoprotein

LFCV = Cardiovascular Physiotherapy Laboratory

LMM = Linear Mixed Models

NC = neck circumference

NUPEF = Research Nucleus in Physical Exercise

PH = Placebo group for TUS+Aussie associated with HIIT

PSQI = Pittsburgh sleep quality

RRi = RR intervals

SAP = Systolic Arterial Pressure

SAT = Subcutaneous Adipose Tissue

SPIRIT = Standard Protocol Items for Clinical Trials

TC = Total Cholesterol

TUS = therapeutic ultrasound

UFSCar = Universidade Federal de São Carlos

usCRP = ultrasensitive c-reactive protein

VLDL = Very Low-Density Lipoprotein

WC = waist circumference

WHR = waist-hip ratio

DATA AVAILABILITY

The data will be collected on paper and stored in folders at the LFCV (UFSCar). The data will be entered into Excel software by the physiotherapist responsible for the assessments, reassessments and follow-ups. In addition, all the data will be available on a public research bench.

The database and electronic analyses will be stored on a secure computer server, with personal login access authorized by the principal investigator of this study. The principal investigator will have access to the complete data set (without the allocation groups), and the data and materials in this article will be available to other researchers on request. Upon completion of the study, all data and documents from the study will be archived by the principal investigator of this study for five years at the Physiotherapy Department of the Federal University of São Carlos.

AUTHOR CONTRIBUTIONS

ACAMS: conceptualization, methodology and writing of the original version. PR-S, EFS, AC, CCD and LF: methodology and review. REL, AMC: conceptualization, methodology, review and

supervision. AMC: acquired funding. All read and reviewed the final version. All authors have read and approved the final manuscript.

PUBLISHER'S NOTE

Not applicable.

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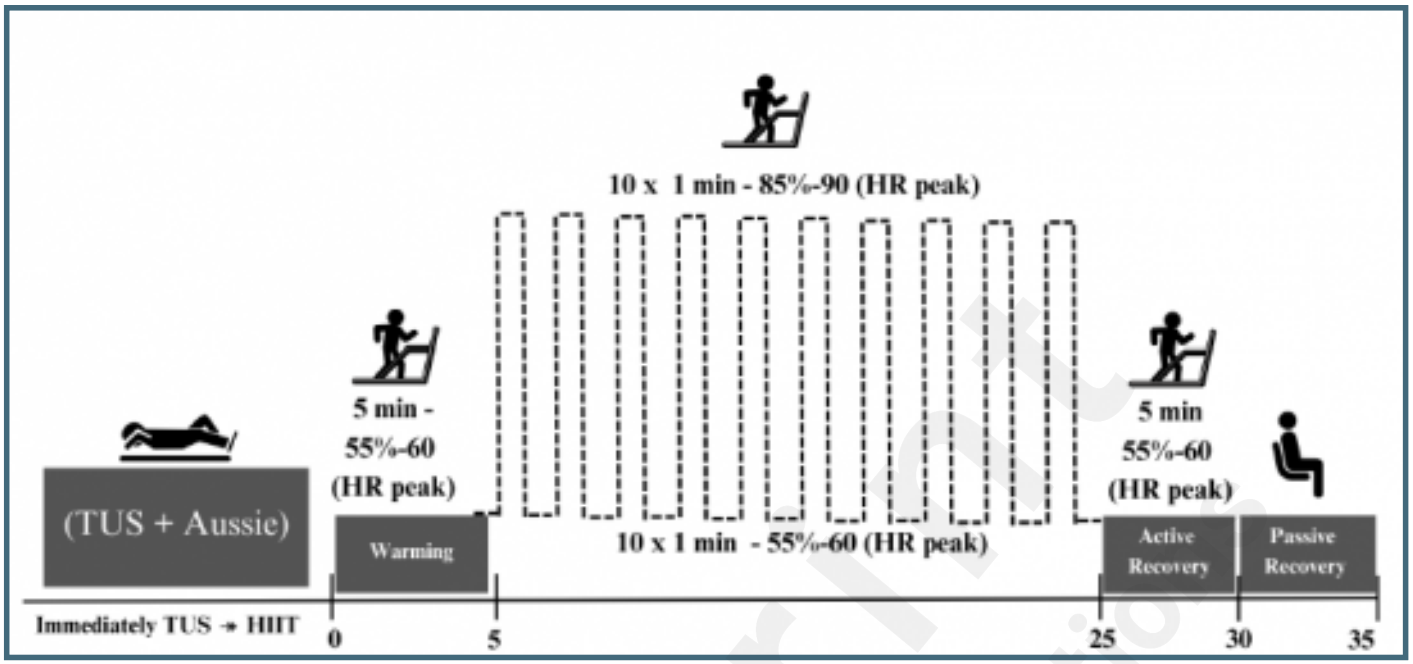
Supplementary Files

Figures

Untitled.



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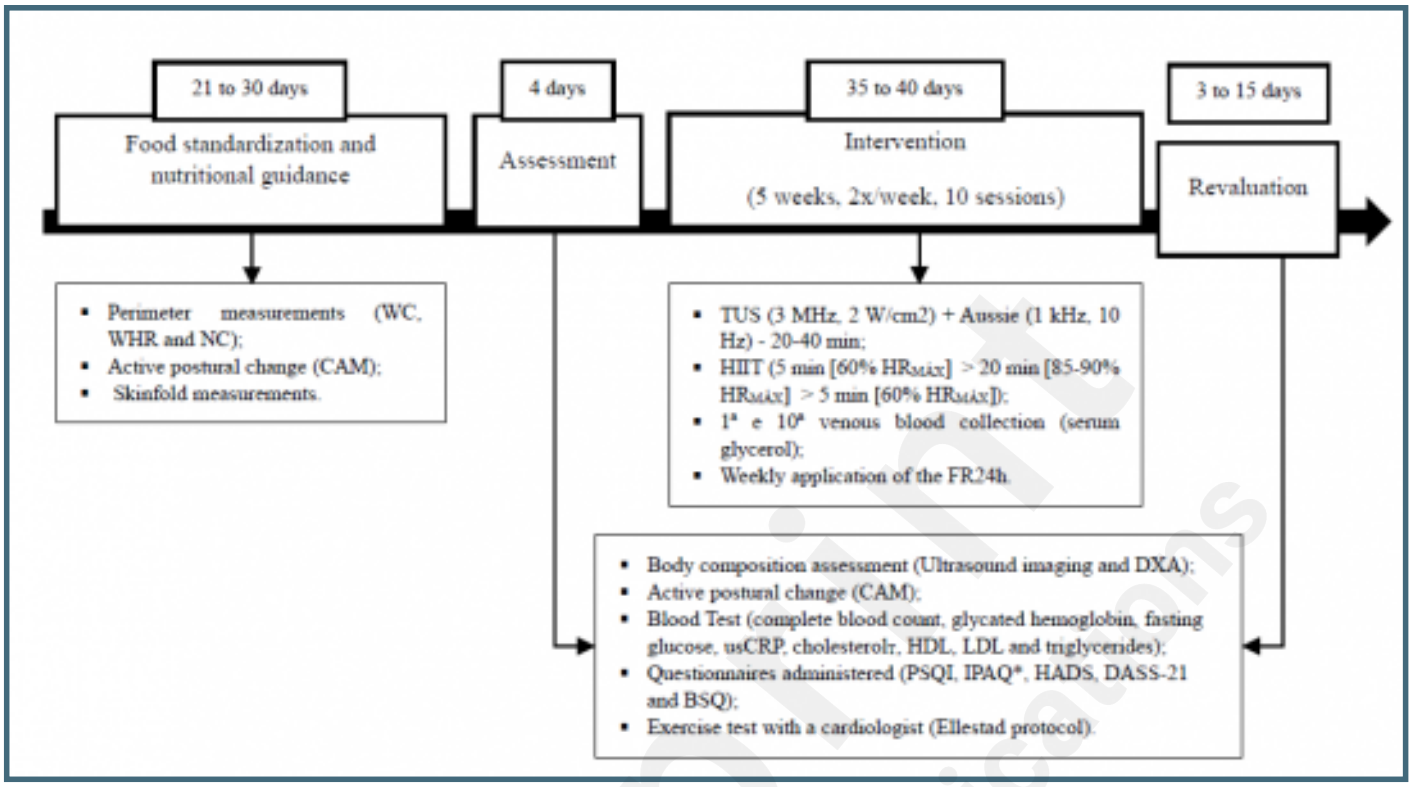

CONSORT (or other) checklists

Supplementary material.
URL: http://asset.jmir.pub/assets/11252d5de1acb78d2d5a6c0b0bfc20a0.pdf